



ATTACHMENT A

Amendment to the Specification

Please amend the application as set forth in the marked-up version of the paragraphs identified below:

Please replace the paragraph beginning at Page 10, line 9 with the marked-up version as follows:

In carrying out the method of the present invention, the isolation and/or purification of the Map protein or of the Map19 protein, or other active fragments or domains of the Map protein, can be accomplished in a number of suitable ways as would be recognized by one skilled in the art. For example, both the Map protein and the Map19 protein may be produced recombinantly using conventional techniques well known in the industry. With regard to the Map19 protein (SEQ ID NO:2), one such suitable method would be through expression in *E. coli* (e.g., JM101 from Qiagen®, Chatsworth, CA) harboring the appropriate plasmid (11-16). In this method, *E. coli* was grown at 37° C in LB containing the appropriate antibiotics until they reached an A_{600} of 0.6 (17). Isopropyl- β -D-thiogalactopyranoside (IPTG) (Life Technologies) was added to a final concentration of 0.2 mM, and the cells were incubated at 37°C for an additional 4 hours. Cells from a 1 L culture were harvested by centrifugation and resuspended in 10 ml "binding buffer" (BB) (20 mM Tris HCl, 0.5 M NaCl, 15 mM imidazole, pH 8.0) and lysed in a French pressure cell at 11,000 pounds/inch² (13). The lysate was centrifuged at 40,000 x g for 15 min and the supernatant filtered through a 0.45 µm filter. A 1 ml iminodiacetic acid Sepharose column (Sigma, St. Louis, MO) was charged with 75 mM NiCl₂·6H₂O and equilibrated with BB. The filtered supernatant was applied to the column and washed with 10 volumes of BB, then 10 volumes of BB containing 60 mM imidazole. The bound proteins were eluted with BB containing 200 mM imidazole, dialyzed against PBS containing 10 mM EDTA, then dialyzed against PBS (13). Protein concentrations were determined by the Bicinchoninic Acid (BCA) Protein Assay (Pierce) and proteins were stored at -20°C until use.

Please replace the paragraph beginning at Page 28, line 14 with the marked-up version as follows:

Expression and Purification of Recombinant Proteins

Recombinant Map19, DbpA SdrF, M55, CNA, ACE19 and ACE40 were expressed in *E. coli* (JM101) (Qiagen®, Chatsworth, CA) harboring the appropriate plasmid (11-16). *E. coli* was grown at 37° C in LB containing the appropriate antibiotics until they reached an A_{600} of 0.6 (17). Isopropyl- β -D-thiogalactopyranoside (IPTG) (Life Technologies) was added to a final concentration of 0.2 mM, and the cells were incubated at 37°C for an additional 4 hours. Cells from a 1 L culture were harvested by centrifugation and resuspended in 10 ml "binding buffer" (BB) (20 mM Tris HCl, 0.5 M NaCl, 15 mM imidazole, pH 8.0) and lysed in a French pressure cell at 11,000 pounds/inch² (13). The lysate was centrifuged at 40,000 x g for 15 min and the supernatant filtered through a 0.45 µm filter. A 1 ml iminodiacetic acid Sepharose column (Sigma, St. Louis, MO) was charged with 75 mM NiCl₂·6H₂O and equilibrated with BB. The filtered supernatant was applied to the column and washed with 10 volumes of BB, then 10 volumes of BB containing 60 mM imidazole. The bound proteins were eluted with BB containing 200 mM imidazole, dialyzed against PBS containing 10 mM EDTA, then dialyzed against PBS (13). Protein concentrations were determined by the Bicinchoninic Acid (BCA) Protein Assay (Pierce) and proteins were stored at -20°C until use.